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Research Note

Growth of *Naegleria* with Insulin

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ABSTRACT: The growth factor insulin was added to axenic cultures of pathogenic *Naegleria fowleri* and nonpathogenic *Naegleria gruberi* to determine whether it would enhance the growth of amebae. The growth of *N. fowleri* was not affected by the presence of insulin; however, *N. gruberi* was inhibited by 100 μg/ml insulin. Based on these results, *N. fowleri* could tolerate the levels of insulin in the brain and especially that of the olfactory bulbs, the tissues invaded, which have much higher concentrations of insulin than the serum.

KEY WORDS: *Naegleria fowleri, Naegleria gruberi,* insulin, growth, culture.

Naegleria fowleri Carter, 1970 is a pathogenic free-living ameboflagellate and the cause of a fatal human disease known as primary amebic meningoencephalitis (reviewed by John, 1993). *Naegleria gruberi* Schardinger, 1899 (Fulton, 1970), a nonpathogenic relative of *N. fowleri,* is a common and widely distributed ameba in freshwater and terrestrial habitats (Page, 1988). The hormone insulin has been used as a growth promoter with a variety of cell types in culture (Ellis, 1989). The purpose of this study was to determine whether insulin would enhance the growth of *N. fowleri* and *N. gruberi* in culture.

The LEE strain of *N. fowleri,* originally isolated from cerebrospinal fluid by E. Clifford Nelson in Richmond, Virginia, in 1968 (Duma et al., 1971), was obtained from Clifford Nelson. Amebae were grown axenically in Nelson's medium (Nelson and Jones, 1970; for composition see Weik and John, 1977) in 25-cm^2 polystyrene tissue-culture flasks (Corning Glass Works, Corning, New York). Cultures were inoculated with 1×10^5 amebae and incubated at 37°C. The NEG strain of *N. gruberi,* derived from the EG strain (Fulton, 1970), originally isolated from soil by Frederick L. Schuster in Berkeley, California, in 1960 (Schuster, 1969), was kindly supplied by Chandler Fulton. Amebae were adapted to grow at 37°C by gradually increasing the incubation temperature from 30°C over a period of months and were cultivated in Mix ameba medium (John, 1993) in 25-cm^2 tissue-culture flasks (Corning). Mix ameba medium is an equal mixture of Balamuth's (Balamuth, 1964) and Nelson's media. Cultures were inoculated with 1×10^5 amebae and incubated at 37°C. Cell counts were made with a Coulter counter (model Z_{BI}; Coulter Electronics, Inc., Hialeah, Florida) using settings described elsewhere (John and John, 1989).

Insulin, purchased from Sigma Chemical Company (St. Louis, Missouri), was solubilized in 1 M acetic acid and added to Nelson's and Mix ameba media, containing reduced calf serum (1%), at concentrations of 1, 10, or 100 μg/ml. The serum was reduced from 2% in Nelson's medium and 4% in Mix ameba medium in order to make any changes in growth more marked. The control cultures contained medium, 1% calf serum, and 1% acetic acid, the concentration of acetic acid equivalent to that present in cultures containing 100 μg/ml insulin. Triplicate cultures were prepared of each concentration of insulin and of controls.

Figures 1 and 2 show the growth of *N. gruberi* and *N. fowleri,* respectively, in the presence of varying concentrations of insulin. *Naegleria gruberi,* the nonpathogen, was affected by the addition of insulin to the medium. Concentrations of 1 μg and 10 μg/ml insulin had little effect on the growth of *N. gruberi*—1 μg may have slightly increased growth and 10 μg/ml slightly decreased growth—however, 100 μg/ml inhibited growth (Fig. 1). In contrast, the growth of *N. fowleri* was not affected by the addition of insulin to the medium, even at a concentration of 100 μg/ml (Fig. 2).

The working range of insulin in cell culture media is 0.001–20 μg/ml (Sigma, 1991). For example, H-Y medium, a medium specifically designed to support hybrid cells in culture, requires 8.3 μg/ml insulin. Thus, the 2 lower concentrations used in the present study, 1 μg and 10 μg/ml, were within the range of added media supplements. However, these quantities would be considerably higher than the levels of insulin that occur in the blood, approximately 0.008 μg/ml (Dittmer, 1961), and would be considered su-

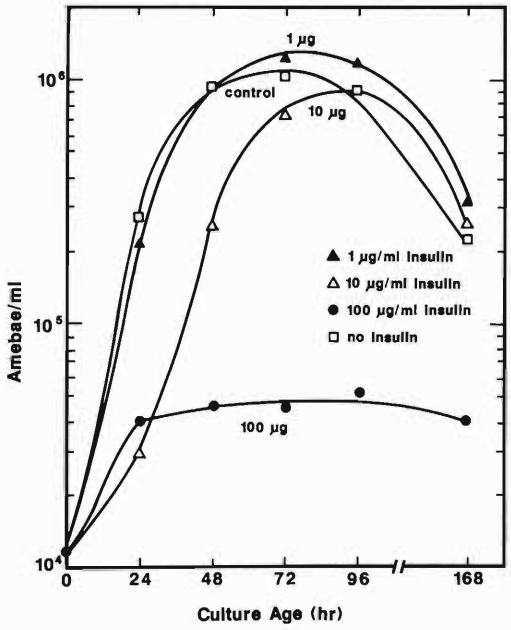

Figure 1. Growth of *Naegleria gruberi* (NEG) in Mix ameba medium with insulin at 37°C. Each point represents the average of 9 counts for triplicate cultures.

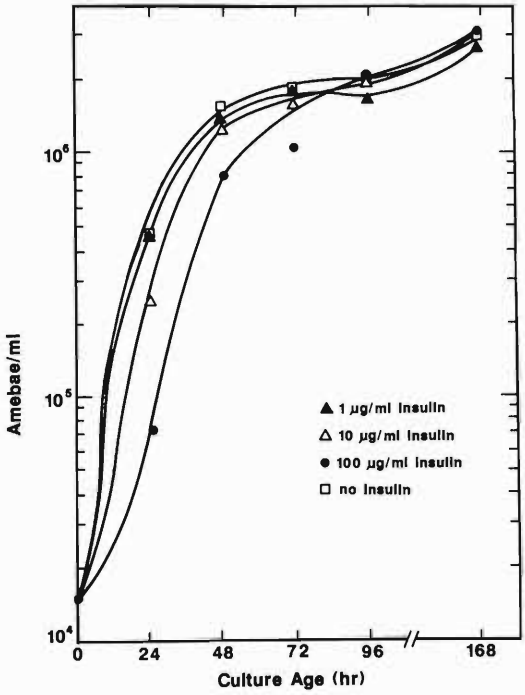

Figure 2. Growth of *Naegleria fowleri* (LEE) in Nelson's medium with insulin at 37°C. Each point represents the average of 9 counts for triplicate cultures.

praphysiological concentrations, as would concentrations greater than 0.1 µg/ml (Koontz and Iwahashi, 1981).

The results presented here show that the growth of *N. fowleri* was unaffected by the addition of insulin to the medium, even at concentrations of 100 µg/ml. Insulin is present in the mammalian central nervous system and in the cerebrospinal fluid (Plata-Salamán, 1991) and there is accumulating evidence for its de novo synthesis by neurons in the brain (Schechter et al., 1992). Experimental studies in the rat have demonstrated that insulin levels in the brain averaged 25 times higher than plasma levels, with the olfactory bulbs and hypothalamus having concentrations 75–100 times higher than plasma (Havrankova et al., 1978). Thus, for an organism such as *N. fowleri* to invade via the nasal mucosa and olfactory bulbs it would have to tolerate substantial concentrations of insulin. Although the growth of *N. fowleri* was not affected by high concentrations of insulin, nonpathogenic *N. gruberi* was inhibited by such concentrations.

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Research Note

Identification of a *Haemonchus placei*-Specific DNA Probe

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ABSTRACT: A partial DNA library was generated from *Haemonchus placei* and differentially screened to identify clones containing repetitive and species-specific sequences. A DNA sequence, which hybridized with *H. placei* genomic DNA by dot-blot analysis and did not hybridize with *Haemonchus contortus* DNA, was identified and characterized. The probe, designated pHp3, was sequenced and found to be 723 bp in length, constituting 0.34% of the *H. placei* genome. The pHp3 probe is useful in differentiating the morphologically similar parasites *H. placei* and *H. contortus.*

KEY WORDS: *Haemonchus placei, H. contortus,* DNA probe, species specific.

Haemonchus is a genus among trichostrongyle nematodes that parasitizes the abomasum of ruminants. The different *Haemonchus* species (9–10 species have been recognized [Gibbons, 1979; Lichtenfels et al., 1993]) develop in a variety of domesticated and wild ruminant hosts, with *Haemonchus placei* and *Haemonchus contortus* being species typically found in cattle and sheep, respectively (see Lichtenfels et al., 1986). Another species, *H. similis,* occurs in cattle especially in southern North America, Central and South America, but it is easily distinguished morphologically from *H. contortus* and *H. placei.* Mixed infections with the 2 morphologically similar species, *H. placei* and *H. contortus,* occur in both domesticated hosts, leading to discussion of the validity of 2 species. Some authors (Gibbons, 1979) have synonymized *H. contortus* and *H. placei* while others (Le Jambre, 1979, 1981; Lichtenfels et al., 1986, 1993) have provided evidence for the recognition of both species. Lichtenfels et al. (1986, 1988, 1993) have described morphological characteristics in detail for identification of individual worms of the 2 species, i.e., cuticular ridge patterns and spicule lengths. These characters make it difficult to differentiate female worms without training and require the recovery of adult worms and hence killing of the host.

In the current report, we describe the development and application of an *H. placei*-specific DNA probe, which can discern *H. placei* and *H.*

Nucleotide data reported in this paper have been submitted to the Genbank™ data base with the accession number: L20568.